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Evaluation of Salt Damage Through Cell Membrane Stability Monitored by Electrolyte Leakage in Water Chestnut (*Trapa* sp.)

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Summary

Cell membrane stability, an evaluator of salt damage in five water chestnut (*Trapa* sp.) varieties viz. Chinese, Japanese, Italian Ⅰ, Italian Ⅱ and Indian was investigated by the measurement of cell electrolyte leakage. The electrical conductance measurement of electrolyte leakage from the leaf pieces stressed by exposure to a solution of polyethylene glycol. Among the different concentrations of polyethylene glycol, 20% solution showed the differences of percent injury clearly. The electrolyte leakage percent was markedly influenced by age of the leaf and sampling time of seasons. With the increased of NaCl concentration, the cell electrolyte leakage percentages were increased in all varieties but the varietal differences were present there. Italian Ⅰ variety showed the highest cell membrane stability. The varieties were overall ranked as : Italian Ⅰ > Japanese > Chinese > Indian > Italian Ⅱ. The results indicated that this technique was effective to assess the salt tolerance in water chestnut species.

Key words : Cell membrane stability, Electrolyte leakage, Salt tolerance, *Trapa*, Water chestnut.

Introduction

Under salt stress condition, plant adaptation and production are complex mechanisms. Many studies point to cell membranes as an initial site of stress injury and drastically damaged by any environmental stress[1-7]. Plaut and Federman[8] reported in cotton, similar symtoms were observed into plant when grown under salt or drought conditions. Commonly, changes in the electrical impedence and electrolyte leakage have been measured to detect stress injury of cell membrane[5]. Leakage will vary in relation to the membrane's abilities to take up and retain solutes and therefore, will reflect stress induced changes in both membrane potentials and membrane permeability[5,9].

This technique is a modification of a method developed by Dexter et al.[10,11] for measuring freezing resistance. Sullivan and Ross[5] have conducted many experiments concerned with the relationship between electrolyte leakage a desiccation treatment and the general ability to the stress as evaluated by electrolyte leakage correlate well with tolerance of other plant process to the stress. To date, this method has been successfully used to measure membrane integrity in plants subjected to a variety of environmental stress[2,3,6,7,9,12]. This method was found to be efficient in estimating stress tolerance of several crops[6,13,14]. However, upto now, no attempt has been made to be established as a measure of salt tolerance in crops by this method.

The objective of this study was to measured the cell membrane stability at successive intervals during the growing season as an evaluation of salt tolerance in water chestnut species.

Materials and Methods

Five varieties of water chestnut viz. Chinese (*Trapa bicornis* Roxb.), Japanese (*T. japonica* Flerov.), Italian Ⅰ (*T. natans* Linn.), Italian Ⅱ (*T. quadrispinosa* Wall.) and Indian (*T. bispinosa* Roxb.) ; grown into the different salt stress condition in the net house of Saga University, were used as materials. Uniform germinated 20 days aged three seedlings of each variety were sown into each pot (50 cm x 30 cm x 30 cm H) containing different concentrations of NaCl solution (0%, 0.1%, 0.2%, 0.3%) on April 25, 1999. Three grams of slow release granular fertilizer containing 16% N, 17% K and 16% P were mixed with the soil of each pot. NaCl concentrations were measured regularly by salt meter (HORIBA, compact salt meter C-121) and water depth (25 cm) maintained by the irrigation of each pot.

For the measurement of cell membrane stability, at 90 and 120 days after treatment, leaf samples were taken from the fully expanded leaves of 7th nodal position of a rosette of each treatment (except leaf age experiment) and were cut into 1 cm^2 pieces. Twenty leaf pieces were put into a 100 ml flask and washed slowly with three changes of deionized distilled water to remove surface adhered electrolytes. Following the washing, the leaf pieces were submerged in 30 ml of polyethylene glycol (PEG) solution for 24 hours at 10℃ in the dark to minimize secondary effects. High molecular weight, solid form of PEG 6000 was used in this study. After the treatment period, the leaf pieces were washed quickly for three times with deionized distilled water. Thirty ml of deionized distilled water were then added and kept for 24 hours at 10℃ in the dark. Then the flask was warmed to 25℃, shaken well and the electrical conductivity was measured by conduct meter (Model CM-2A, TOA Electronics Ltd. Japan). Following the conductivity measurement, the leaf tissues were killed by autoclaving for 15 minutes (121℃ at 1.06 kg cm^{-2} pressure) to release all ions from the tissue, cooled to 25℃ and then the electrical conductivity was measured. Three replicates were measured from each salt treatment (T) and non-treated control (C). Degree of electrical leakage (EL) is evaluated using following equation :

$$EL = \{1-(1-T_1/T_2)/(1-C_1/C_2)\} \cdot 100$$

T_1 = first conductivity measurement,

T_2 = second conductivity measurement,

C_1 = first conductivity measurement of control,

C_2 = second conductivity measurement of control,

A preliminary test was conducted to find the appropriate concentration of PEG solution and from here, cultivars differences were clearly identified used by this concentration. Influence of age of the leaf and the sampling time of seasons on salt tolerance were investigated.

Results and Discussion

For estimating the cell membrane stability, PEG test is long standing method for assessing

environmental stress such as drought, heat, water stress etc. To measure the cell membrane stability using this method in water chestnut grown under different salt stress conditions may assess the salt tolerance. Because, drought and salinity in the root environment of plants are frequently considered as imposing similar effects on plants due to the decrease in external water potentials[8]. This method is based on the measurement of the electroconductivity of aqueous media containing leaf discs that were previously water stressed *in vitro* by exposure to a solution of PEG. Therefore, the values obtained with PEG test have been considered to be influenced by factors in terms of membrane permeability such as leaf surface wax content, thickness of cuticular layer and cell wall, changes in cytoplasmic lipids, and osmotic adjustment[6,7].

In this experiment, firstly, investigated the relationship between PEG concentration and electrolyte leakage of the leaf tissues. Electrolyte leakage increased from 10% to 60% with the increasing of PEG concentrations from 20% to 50% (Fig. 1). For testing drought tolerance in orchardgrass, Premachandra and Shimada[14] used 50% of PEG at which the difference between cultivars was maximum. In sorgam and wheat, 30% and 25% of PEG were used respectively in the same reason[7,15]. The similar degree of leakage was occurred at 20% of PEG in water chestnut grown under different salt stress conditions and the differences of percent injury between cultivars are clearly indicated and are statistically significant (Fig. 1), therefore, this PEG concentration was used as a standard osmotic medium for further work in water chestnut.

Effect of the age of the leaves on salt tolerance is shown that percent injury increased with the increase of the age of the leaves (Fig. 2). Sullivan[16] and Blum[13,17] concluded that the younger leaf tissues are more tolerant to drought than older tissues in wheat and sorghum respectively.

The influence of age of the water chestnut plant on salt tolerance observed that a decrease in percent injury with the increasing of plant age (Fig. 3). After 90 and 120 days of sowing, the percent of injury were significantly different within them and this difference may be due to some

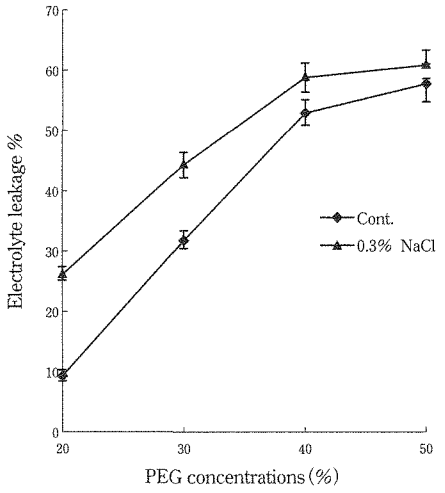

Fig.1. The relationship of PEG concentration and electrolyte leakage % of Chinese control and 0.3% NaCl treated variety after 90 days of sowing.

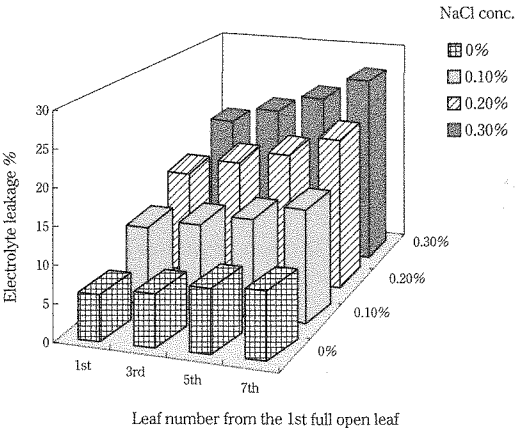

Fig.2. The influence of leaf age on electrolyte leakage % grown under different NaCl conditions of Chinese variety (after 90 days of sowing treated with 20% PEG solution).

physiological changes in plant tissues under two aged conditions. Under drought condition, Premachandra and Shimada[14] found in wheat, a decrease in percent injury with the increasing of plant age.

Through the measurement of cell membrane stability from the five varieties of water chestnut, it was indicated that cell membrane stability significantly decreased to increased the NaCl concentrations. Cultivar differences of five water chestnut on salt tolerance are shown that the percent injury of the Italian I cultivar was the minimum and other water chestnut cultivars showed differences in salt tolerance clearly (Fig. 4). Decreasing rate of growth and leaf morphology was also lower in Italian I variety than other variety under this conditions (Data not shown). From the result it was clear that Italian I variety was more salt tolerant than other variety and followed by Japanese one. Salt tolerance tests using PEG were found to be comparatively efficient in the evaluation of salt tolerance between cultivars. Special care should be taken on age of the leaves, age of the plants and the sampling position of the leaf when obtaining samples for testing, for accurate assessment. Further studies on physiological meanings and genetical basis of the salt tolerance in water chestnut will be carried out by this technique for field studies and breeding programmes.

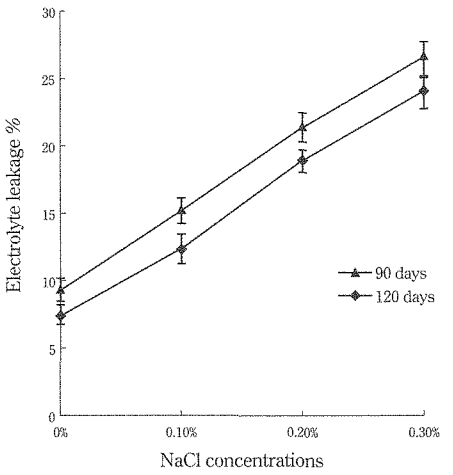

Fig.3. The influence of plant age on electrolyte leakage % of Chinese variety tested with 20% PEG solution after 90 and 120 days of sowing into different NaCl concentration.

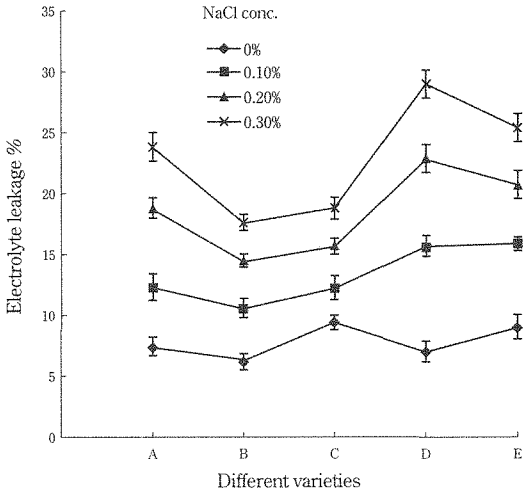

Fig.4. Varietal differences of electrolyte leakage % treated with 20% PEG solution in water chestnut after 120 days of sowing under different NaCl conditions.
A, Chinese ; B, Japanese ; C, Italian I ; D, Italian II ; E, Indian.

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細胞膜安定性を指標としたヒシの耐塩性評価法の検討

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摘　　要

水生作物のヒシ(*Trapa* sp.)における耐塩性の評価法として，ポリエチレングリコール(PEG)試験法の応用を検討した．その結果，ポリエチレングリコールの濃度は20%が適当であり，測定部位とした葉身の細胞膜の損傷程度は，生育時期ならびに測定葉位により大きく変化することが分かった．日本産，中国産，インド産各1種，イタリア産2種の計5種のヒシを異なるNaCl濃度で栽培して細胞膜の損傷程度を測定したところ，損傷程度はすべてのヒシでNaCl濃度の上昇に伴って高まり，イタリア産Ⅰ＜日本産＜中国産＜インド産＜イタリア産Ⅱの順で生育状態に対応した種間差異が認められた．これらのことから，本方法がヒシにおいても耐塩性の検討に有効であることが示唆された．